

The South African Journal *of* Medical Laboratory Technology

ORGAN OF THE SOCIETY OF MEDICAL LABORATORY
TECHNOLOGISTS OF SOUTH AFRICA

Vol. 3, No. 2

A QUARTERLY

March, 1957



EDITOR:
CECIL R. STUART

Single Numbers 5/6

Annual Subscription £1/1/-

**SOCIETY OF MEDICAL LABORATORY TECHNOLOGISTS
OF SOUTH AFRICA**

— o —

President: Dr. J. C. THOMAS

Vice-Presidents:

Prof. J. BARNETSON Prof. I. GORDON

Dr. R. ELSDON-DEW Prof. J. F. MURRAY

Dr. R. TURNER

— o —

COUNCIL

Chairman: Dr. F. A. BRANDT

Mr. J. MAYTHAM Mr. A. SCOTT

Mr. G. TURNER Mr. P. ROUX

Mr. V. ALBERTO Mr. G. C. BUCKLE

General Secretary-Treasurer: Mr. G. W. WIKLEY

— o —

BRANCH SECRETARIES

Natal: Mr. V. ALBERTO **Cape:** Mr. J. MAYTHAM

Southern Transvaal: Mrs. G. TOMLIN

— o —

Editor: CECIL R. STUART

Editorial Committee

Mr. P. N. BUCK Mr. R. HORNER

Mr. W. G. POWELL Miss I. E. MULLER

Mr. A. J. S. GREENFIELD

Clerical Assistants:

Miss P. M. EDROP Miss F. M. MOTTRAM



Accuracy

In laboratory work accuracy and perseverance are two principles. Perseverance can only be achieved; accuracy, however, can be obtained.

From our delivery programme:

ZEISS Microscopes for every purpose including two automatic Photo Microscopes and a unique Micro Cine Camera

ZEISS Spectrophotometers, Colorimeters and Polarimeters of most modern design

ZEISS Ophthalmological Instruments and Operating Microscopes

SCHOTT JENAer Glassware of world fame

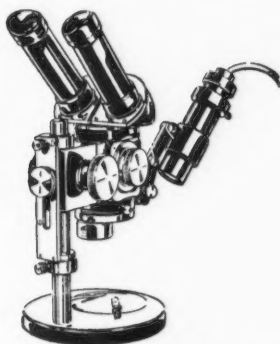
HERAEUS Electrical Laboratory appliances of highest standard

R. JUNG Microtomes for every laboratory

ELLIOTT Tissue Processing Equipment

SPOERHASE Balances of repute

HETTICH Laboratory Centrifuges



ZEISS Stereomicroscope



HERAEUS Crucible furnace

Optical Instruments (Pty.) Ltd., Maritime House, Johannesburg

97a Main Street, P.O. Box 1561 Tel. 34-3980/88/89
Sole ZEISS Representatives for the Union and the Central African Federation.



ARE YOU INTERESTED IN ANIMALS WITHOUT BEING A FANATIC?

Then write for the illustrated prospectus of

UFAW

(The Universities Federation for Animal Welfare)

7a Lamb's Conduit Passage, London, W.C.1

UFAW fosters consideration for the physical and mental comfort of experimental animals, the avoidance of procedures which involve serious suffering, and the development of techniques calculated to reduce discomfort to a minimum.

Publications include:—

The UFAW Handbook on the Care and Management of Laboratory Animals, edited by A. N. Worden, M.A., B.Sc., M.R.C.V.S., F.R.I.C. (Baillière, Tindall & Cox, 31s. 6d.).
Kind Killing, compiled by F. Jean Vinter, M.D., Practical instruction in euthanasia for animals. Enlarged 3rd edition, 6d.

How to Befriend Laboratory Animals, by C. W. Hume, M.C., B.Sc., 3d.
Suggestions for the Legal Protection of Laboratory Animals Overseas, gratis.

ANNUAL MEMBERSHIP FEE: Technicians 5/-; Student Technicians 1/-

SWAB WIRES

★ LARYNGEAL

★ NASAL

★ THROAT

★ UTERINE

All types and descriptions manufactured

Send for Price List and Samples

MEDICAL WIRE & EQUIPMENT CO. (BATH) LTD.

27 Charmouth Road — — Bath, Somerset — — England

Stability!

AMIDST THE SEA OF RISING COSTS CONSIDER GURR'S
RECORD IN MICROSCOPICAL STAIN PRICES.

	1919 list	Current list
Methylene blue 10 g.	2/9	2/6
Leishman stain 5 g.	8/-	3/-

TWO EXAMPLES FROM MANY

Growing demand has enabled us generally to whittle prices—and you have shared the benefit of nearly 40 years.

Please forgive us this dissertation on our own efficiency—Your confidence and support have been vital, have always been appreciated, and will enable us to continue to bring you the best. Our slogan in 1919—
"Stipulate Gurr's"—is still the best advice.

Ask for Catalogue E 56



GEORGE T. GURR LTD.

136/138 NEW KINGS ROAD - LONDON, S.W.6

Sole Agents for the Union of South Africa . . . B. Owen Jones Limited

JOHANNESBURG

CAPE TOWN

DURBAN

P.O. Box 9955

P.O. Box 434

P.O. Box 557

STUART JONES & DAVID ANDERSON LTD.

20 QUEEN STREET - DURBAN

•

WE ARE PROUD OF THE FACT THAT WE HAVE SUPPLIED MUCH
OF THE EQUIPMENT NOW IN USE IN THE LABORATORIES OF
THE NATAL PROVINCIAL ADMINISTRATION

*We extend a cordial invitation to visit our showroom at the above
address*

Natal Agents for-

Baird & Tatlock (London) Limited, W. Edwards & Co. (London) Limited,
Hilger & Watts Limited, Hopkin & Williams Limited, Watson & Sons
Limited, George T. Gurr. Limited

DADE REAGENTS, INC.

The standard of excellence the world over for

BLOOD GROUPING AND TYPING SERUMS—The pioneer line of stable, specific, fast-acting serums.

HEMOLET^R—The pioneer sterile, expendable, stainless steel finger puncture lancet.

LAB-TROL^R—The pioneer stable, ready-to-use control solution for blood chemistry tests.

ALBUMIN—Specific, reagent grade albumin made solely for laboratory use.

CATH-URINE^R—Sterile, expendable combination catheter and specimen container.

Other specialty items for **BLOOD BANKS, HOSPITALS AND CLINICAL LABORATORIES.**

PROMPT AIR SHIPMENTS

Suppliers to governments of the United States and many overseas countries.



INQUIRIES INVITED



DADE REAGENTS, INC.

1851 DELAWARE PARKWAY

MIAMI 35, FLORIDA

Phone : NEWton 4-1581

Cables : DRINC-MIAMI

The South African Journal *of* **Medical Laboratory Technology**

ORGAN OF THE SOCIETY OF MEDICAL LABORATORY
TECHNOLOGISTS OF SOUTH AFRICA

Vol. 3, No. 2

A QUARTERLY

March, 1957

CONTENTS

	<i>Page</i>
Editorial	2
Redaksioneel	3
Collecting, Preserving and Mounting Entomological Specimens ...	4
Drug—Host—Parasite Relationship in the Relapse Phase of Typhoid Fever	9
Paper Electrophoresis of Serum Proteins in Clinical Investigation	13
Eldon Cards	20
The Subterranean Termite	23
Abstracts	24
Pathologist's Letter	26
Readers Forum... ..	28
The Central African Association of Medical Laboratory Tech- nologists	31

SUPPORT THE FIRMS WHO SUPPORT YOUR SOCIETY'S JOURNAL

EDITORIAL

It is felt that a vote of thanks should be accorded to the officers of the Society who, at a recent high level discussion in Johannesburg, helped formulate an educational policy which, if brought into fact, would assure a bright future for Medical Laboratory Technologists in South Africa.

It is well known that there is a shortage of trained technologists both here in South Africa and overseas. There is a trend with certain employing authorities to alleviate this shortage by engaging university graduates to fill posts established for technologists. There is of course no reason why this should affect the technologists provided that these graduates are employed as a temporary measure and also that they are graded according to their practical experience of Medical Laboratory Technology. There are exceptions where the graduate's education fits them for a specialist post in a clinical laboratory. In this case the employing authorities should recognise the fact and employ them as scientific officers.

There can be no question of a B.Sc. graduate majoring in one subject which has a connection with one specialist division of Medical Laboratory Technology being considered equivalent to a trained technologist with experience in all six subjects. The untrained graduate cannot be as generally useful as the trained technologist in the routine clinical laboratory.

The realisation that a university graduate employed as a technologist would have preference by virtue of his academic background, for promotion must be faced. This would tend to block trained technologists from senior posts. There may be a place for the university graduate in the routine clinical laboratory, but he should not be graded as a technologist unless he is prepared to undergo training in the subjects of which he has no knowledge. When he is proficient in all divisions of medical laboratory technology he should be graded "technologist".

REDAKSIONEEL

Daar word gevoel dat 'n woordjie van dank verskuldig is aan die beamptes van die vereniging wat op 'n onlangse besonder belangrike vergadering in Johannesburg, die opvoedkundige belied help preuleer het, indien hierdie belied ten uitvoer gebring word, verseker dit 'n rooskleurige toekoms vir Mediese Tegnoloë in Suid-Afrika.

Dit is alom bekend dat daar 'n tekort aan opgeleide tegnoloë hier in Suid-Afrika, asook in die buiteland is. Die neiging bestaan egter om hierdie tekort aan te val met mense in besit van universiteitsgrade. Indien hierdie persone slegs as tydelike werknemers aangestel word en ook op voorwaarde dat hulle geklassifiseer word volgens hulle praktiese ondervinding van die mediese laboratorium tegnologie, behoort die toestand die tegnoloë geensins te affekteer nie. In gevalle waar die persone met universiteitsopleiding geskik is vir 'n spesialiteitspos, behoort die gesaghebbendes hulle as wetenskaplikes in diens te neem.

Die persoon met 'n B.Sc. graad kan onmoontlik gelykstaande wees aan 'n opgeleide mediese tegnoloog met ondervinding in al ses vakke waar sy—die B.Sc.—vak slegs een opleiding van die mediese laboratorium Tegnologie dek. Die B.Sc. sal ook oor die algemeen in die roetine laboratorium minder bruikbaar wees as die opgeleide tegnoloog.

Die feit dat universiteitsopgeleides terwille van hulle akademiese agtergrond voorkeur sal geniet bo die opgeleide tegnoloog, wat bevordering tref, en sodoende die weg tot senior poste vir tegnoloë versper, moet in aanmerking geneem word.

Daar mag wel 'n plek in die roetine laboratorium vir die universiteitsopgeleide wees, maar sal nie as tegnoloog geklassifiseer word nie, tensy hy bereid is om so 'n opleiding te ondergaan. Slegs wanneer hy hom bekwaam het in al die afdelings van die mediese laboratorium tegnologie sal hy as „tegnoloog” geklassifiseer word.

COLLECTING, PRESERVING AND MOUNTING ENTOMOLOGICAL SPECIMENS

W. G. POWELL

Department of Bacteriology, Government Laboratory, Durban

Medical entomology plays a very important role in the fields of human and animal health. A study of this subject can be of great value and interest to persons concerned with medical science.

A collection of relevant material is the best way of beginning a study of this nature. In order to make the collection it is necessary to know how and where to collect, prepare and store the specimens.

It is not always possible to collect personally, enough specimens for a complete series, although to learn the habits of the insects and to give a greater interest to this study, it is better for the collecting and preparing to be done by one's self. Where difficulty is experienced in obtaining specimens, an approach to workers in entomology, in museums, universities, etc., is usually of great help, not only in obtaining the odd specimens, but also in the identification of material on hand. This is a specialised field and should be entrusted only to entomologists within that field.

There are various methods of preparing specimens for a collection, a brief explanation of some of these methods follows.

Minute specimens such as lice, small ticks, fleas, small flies, etc., which are best mounted on slides, should be collected in 70% alcohol. Larger specimens for pinning, should be collected in cyanide killing bottles.

To make killing bottles, use small screw-capped bottles for small specimens and honey-jars or fruit jars for larger specimens. On the bottom of the bottle or jar, spread a $\frac{1}{4}$ -inch layer of sodium cyanide, cover this with a $\frac{1}{2}$ -inch layer of sawdust and over this an even, thin layer of Plaster of Paris, care must be taken that the cyanide does not become wet, if this happens the killing effect will not last very long. After covering the sawdust with Plaster of Paris, the receptacle must be left open for about two hours to dry, and after this must only be opened when necessary. There are other methods for making killing jars, but the above appears to be the most effective and lasting.

For mounting specimens on slides, the following procedure is recommended. The specimens are transferred from the 70% alcohol in which they are collected, to 95% alcohol for about 15 minutes, the

dehydration is completed in absolute alcohol and the specimen cleared in xylol for about 15 minutes before mounting. In the case of very delicate specimens, it may be necessary to take it from absolute alcohol into a mixture of equal volumes of absolute alcohol and xylol before transferring to pure xylol. This will prevent shrinking and collapsing. While going through the alcohols and xylol, it is necessary to keep the appendages of the specimens more or less in the desired positions. By the time they have gone through the xylol they are fairly hard and easily broken. These appendages can be teased into position with a firm, sharpened bristle from a hair brush, mounted on a suitable handle. Canada balsam is spread on a slide in a sufficient volume to cover the area under the cover-slip, the specimen is then lifted out of the xylol by floating it onto a small artists' brush and transferred to the Canada balsam on the slide. The appendages are carefully teased into position by means of the bristle. A cover-slip is carefully placed over the specimen so that the specimen does not float out or allow air bubbles to remain underneath. If sufficient balsam has been placed on the slide, then more can be added by flowing it in from the side of the cover-slip wherever balsam is lacking. It will flow readily. It is important that legs, wings, mouth-parts, antennae, etc., should be in a position where they can be studied under the microscope for identification purposes.

Specimens which are too large for slide mounts and too small or fragile for pinning may be mounted on stiff cardboard or celluloid. A transparent gum, such as Gum Tragacanth is spread over the square and the specimen taken from the killing bottle or alcohol and placed on the gummed card in the required position and allowed to dry. A pin is passed through one end of this card to support the mount in the store-box or drawer. Mosquitoes, etc., can be pinned with "miniature pins". These are very thin and short. This pin is passed through the thorax of the insect and then through one end of a polyporous or similar soft strip cut to the required size. A large insect pin is passed through the end of this strip to support the mount. This pin will also serve to hold the identity and locality labels.

Large insects are pinned through the thorax. For *Coleoptera* (beetles) it will be found that in most types the thorax is too hard for a pin to go through. It has become a general rule that all specimens of this order are pinned through the right wing, near the thorax. In *Lepidoptera* (moths and butterflies) it is necessary, for appearance and also for identification, that the wings be spread out in order that, in the finished preparation, the wings will be arranged neatly and evenly on the sides of the body. For this purpose the insect is mounted on a block with a groove just wide enough to fit the body and to allow the wings to be spread out and arranged on an even plane over the flat surface of the board, in the desired position. In this manner both rear and front wings can be examined for identification purposes. The best method for spreading the wings is to pin a narrow strip of strong paper

or preferably, blue print tracing linen, over the wings next to, and parallel with the body of the insect.

By holding down the strip on the wings they can be manipulated and controlled by pressing down or releasing with the finger. This allows the operator to hold or release the wings while manipulating them into position. This should be done with a fine pin, held against a vein to prevent damaging the wings during this operation. When in position, the lower end of the strip is also fixed down with a pin, and a wide strip of the same material is placed over the rest of the wings to prevent the tips from curling up while drying. When quite dry, the specimen is removed from the setting board, labelled and placed in an insect store-box or drawer. At no time must the specimen be touched with the fingers, as this will remove the fine scales covering the wings and body.

For pinning other insects, where spreading of wings is not necessary. The specimens are pinned down on a board which is soft and deep enough to allow the pin enough penetration for the legs and underside of the insects to rest on the surface of the board. The appendages may then be spread out in the most suitable positions and other parts of the body can also be held in position by means of pins until dry. When dry, the specimen is taken off the pinning board, labelled and stored.

Instead of pinning insects, a "Riker Mount" can be made. In this method specimens are arranged on cotton wool in a shallow box and covered with glass. These mounts are easily handled, stored and demonstrated without the greater risk of breakage as in the case of pinned material. For this type of mount the specimens are prepared as above. Instead of a pin being passed through the insect they are crossed over the body so as to hold it down without puncturing. This could be done on a "pinning board" or, where wings are to be spread, on a setting board. A shallow box is filled with cotton wool and the dried specimens are placed in hollows in this cotton wool. When the specimens are set out in the desired positions and labelled, a glass cover is placed over them gently pressing them down into the cotton wool. The lid is then fixed with *passe partout* binding. A useful box for this purpose is an hosiery box, approximately one inch deep. The lid can be cut to form a frame for the glass. Naphthalene or preferably, paradichlorobenzene should be spread under the cotton wool to keep out insect pests which will destroy the specimens. It is necessary to keep one of the above insecticides in all boxes and drawers used for storing insect specimens, no matter how well closed these may be. Permethos and other insect pests soon destroy a collection if these precautions are not taken.

Insects in any collection have no scientific value unless they are labelled with the locality in which they are caught, the date collected and the name of the collector. This information should be printed on a small label and placed on the pin under the specimen or next to it in the case of Riker Mounts.

Special rustless steel or black japanned insect pins must be used for pinning insects. Ordinary pins corrode in a very short while if used for this purpose.

Uniformity should be aimed at when making a collection. It should be decided what height the pin should protrude above the insect, what position the locality and identification labels should occupy, before starting the collection.

Specimens should be arranged in the boxes or drawers under their relevant orders, families, genera and species. Life history sets, i.e., the various stages of development from the egg to the adult make a collection even more interesting. Some larvae, caterpillars and various nymphal stages of insects cannot be kept dry unless properly prepared. A suitable method is to boil them gently in a concentrated solution of mercuric chloride for about 15 minutes. Take them through various increasing strengths of alcohol to absolute alcohol, repeat the latter to be sure of thorough dehydration, then transfer to ether for about 24 hours to remove fatty substances. Specimens are taken out of the ether and allowed to dry rapidly. They can then be pinned, gummed on to cards or placed on cotton wool. This method is particularly suitable for white or ivory coloured larvae etc. Large caterpillars, particularly coloured ones, should have their pulp removed by making an opening posteriorly and rolling the contents out with a test tube until quite flat. A tube drawn to a point is inserted into the opening and tied. The specimen can then be inflated while being held over a hot plate to harden. Wire can be glued into the hole and twisted round a pin for support in a store-box or drawer. If it is required to mount a specimen which has become hard and dry, even after some years, it is possible to relax it as follows.

Spread a layer of sand about $1\frac{1}{2}$ to 2 inches deep in the bottom of a jar with a tight fitting lid, mix in a few crystals of phenol to prevent fungal growth, place a layer of blotting paper over the sand and wet thoroughly. Place the specimen in the jar, keeping it clear of the wet surface, close the lid tightly and leave standing until the specimen is sufficiently relaxed. If the weather is cold relaxation could be speeded up by placing the jar in an incubator.

Insects are self-preserving if properly dried but some large species with soft abdomens, this includes other Arthropods such as spiders, etc., should be cut open and the contents of the abdomens replaced with cotton wool which prevents shrinking and discolouring.

Many species of insects can be collected at a strong light at night. The light is so arranged as to shine on to a white wall or cloth. The insects will settle on this and can be taken off by means of a cardboard cylinder, open at one end and covered with glass on the other. When placed over the insect a card can be slipped underneath in order to trap the insect

in the cylinder. It can then be transferred to a cyanide or other killing bottle. The cylinder can be made so as to fit neatly into the top of the jar.

There are many alternative methods of collecting insects: nets, light traps etc. However if one knows where to find the specimen there is always a means of catching it without damage.

VACANCY

Factory Medical Officer in Durban, requires trained or partly trained Laboratory Technician for full or part day, for clinical laboratory work.

Reply in writing to Dr. D. Lapping, Box 21, Merebank, or telephone 81683 in afternoon.

MICHROME STAINS AND REAGENTS

for Microscopy and Biology

Adonitol	Dextrin	Leishman Stain	Sudan Blue
Arabinose	Field's Stain	May Grunwald Stain	Sudan 1, 2, 3, & 4
Asparagine	Gelatin	Methyl Red	Thioflavine, T & S
Auramine, O	Giemsa Stain	Peptone	Thionin
Azur 1, 2, B. C. & L	Glucose	Resazurin	Toluidine Blue
Azur 2 Eosin	Haematoxylin	Rosolic Acid	Urease
Beef Extract	Inositol	Sodium Desoxycholate	Vital Red
Brom Paeonol Red	Janus Green, B	Sodium Taurocholate	Yeast Extract
Cedarwood Oil	Lacmoid	Sodium Thiolglycollate	etc.

Water Soluble Indicators Buffer Tablets Optoil: synthetic, non-sticky immersion oil
Cristallite and Clearmount: Synthetic, colourless, neutral mountants

Microscopic Stains in tablet form

Water Testing Tablets and Outfits Stains for fluorescence microscopy

UNIFORM HIGH QUALITY, RELIABILITY AND IMMEDIATE DELIVERY

55-page Price List of Michrome Stains and Reagents available on request.

EDWARD GURR, LTD.

42 UPPER RICHMOND ROAD WEST, EAST SHEEN, LONDON, S.W.14

Telephone: PRospect 8051 & 7066

Cables: Michromlabs, Put., London

Now ready: "Practical Manual of Medical and Biological Staining Techniques" (2nd edition 460 pages; price 42/-) by Edward Gurr, author of "Microscopic Staining Techniques," Nos. 1, 2 and 3.

DRUG—HOST—PARASITE RELATIONSHIP IN THE RELAPSE PHASE OF TYPHOID FEVER

KENNETH C. WATSON,

Department of Pathology, University of Natal

In most bacterial, rickettsial and viral infections in man, recovery of the host is associated with the development of specific immune antibody. In the case of antitoxic immunity, as in diphtheria and tetanus, such specific antibody is the most important defence mechanism. Similarly, in many virus infections recovery can be shown to be dependent on the production of sufficient amount of protective antibody. In the case of antibacterial immunity however, the role of specific antibody is, in many cases, less certain. Here phagocytic mechanisms of defence are often more important although these may be enhanced by the presence of specific antibody. In the case of typhoid fever it is well known, as example, that flagellar-type antibody has no protective function whatsoever. Somatic-type antibody on the other hand does have some protective effect but it is by no means certain whether recovery in typhoid fever is more dependent on the production of somatic antibody or on other factors such as phagocytosis. The demonstration of bacterial agglutinins, as performed in the Widal test, is no real indication of the state of immunity and the fact that relapses are common in typhoid fever, in spite of the production of antibody, suggests that the antibody produced in this disease is not as fully protective *in vivo* as is the antibody produced in certain other diseases.

Clinical relapses in typhoid fever occur in some 6–10% of untreated cases and some patients may relapse more than once. In many of these it is possible to show that the levels of circulating antibody are very high at the time of relapse. Since the introduction of chloramphenicol in the treatment of typhoid fever, it has become apparent that the incidence of relapse has not been reduced by this form of therapy, in spite of a considerable reduction in the mortality rate and an increase in the speed of recovery as compared with untreated cases. In actual fact it has been found that where patients are treated for an inadequate period of time with chloramphenicol the incidence of relapse may be considerably higher than in untreated cases. In some series of cases the relapse rates have been of the order of 60% where treatment has only been continued for 3–4 days.

RELAPSE PHASE IN TYPHOID FEVER

Before considering the effect of chloramphenicol on the relapse state something must be said about the occurrence of the relapse phase itself. Comparatively little is known as to why certain patients will exhibit a relapse and others not. It appears probable however, that foci of organisms exist in the reticulo-endothelial tissues of the body,

especially in the spleen and liver and that these organisms have the ability to reinvade the blood stream and, if present in sufficient numbers, to cause relapse in spite of the presence of high antibody levels. Some support for this observation is to be found in the fact that it is sometimes possible to isolate the causative organism from the blood stream during the phase of convalescence when the patient is afebrile. In such cases it would seem that there is probably an intermittent shedding of organisms into the blood stream from the reticulo-endothelial tissues but not in sufficient numbers to overcome the body's defence mechanisms. When larger numbers of organisms are released in this way however, then relapse may occur. It also appears probable that the organisms present in the reticulo-endothelial tissues exist in a viable state inside the tissue cells. Typhoid fever in fact has certain features in common with such diseases as tuberculosis and brucellosis where intracellular parasitism is part of the natural history of the illness. Incidentally it has been shown as long ago as 1916 by Rous and Jones that intracellular forms of *Salmonella typhi* are protected from the action of specific immune antibody.

THE EFFECT OF CHLORAMPHENICOL ON THE RELAPSE PHASE

The fact that chloramphenicol may lead to markedly increased relapse rates if not given in adequate amounts has proved to be one of the disappointing features of this antibiotic. Experience has shown that the incidence of relapse tends to increase the shorter the course of treatment. In practice it is essential to continue treatment for at least ten to fourteen days if relapse rates higher than the usual 8-10% are to be avoided. The duration of treatment appears to be more important than the total dosage and nothing is to be gained by the use of large initial loading doses.

The effect of chloramphenicol on the relapse state is not as yet clear but several possibilities can be considered. It has been suggested that the antibiotic, by eliminating numbers of the causative organism, leads to a failure of the immune response due to lack of sufficient antigenic stimulus. This is almost certainly true in the case of certain illnesses such as scrub typhus. Smadel (1954) for example has shown that if patients with scrub typhus are left untreated for several days till the immune response mechanism has come into play, no relapses will occur if chloramphenicol is then given. However, if treatment with chloramphenicol is begun during the first two or three days of illness, then fairly high relapse rates may be found and are associated with very low antibody levels. Depression of antibody production in this way has been demonstrated in other diseases. In the case of typhoid fever however, it seems very unlikely that this is the explanation of the relapse state. It is true that if patients with typhoid are treated in the first few days of illness there is a delay in the production of antibody. However, in an area such as this, it is not usual to find patients admitted to hospital with a history of illness of less than about seven days, by which time the

antibody forming tissues are already producing large amounts of specific antibody. An investigation of some twenty relapse cases in this area (Watson, 1957) has shown that in each case the level of somatic "C" antibody was 1/640 or greater at the onset of the relapse phase. It appears probable that the type of antibody produced in rickettsial infections, such as scrub typhus, is considerably more protective than typhoid antibody.

The problem arises then of why the addition of a suppressive agent such as chloramphenicol should, under certain circumstances, increase rather than decrease the rate of relapse in typhoid fever. A second possible mechanism which suggests itself is that antibiotics may have a direct effect on the antibody forming tissues. A great deal of conflicting literature is available on this point. Some authors have been unable to show any failure of antibody formation in the presence of antibiotic. Stevens (1953) on the other hand, using radio-iodinated bovine gamma globulin as antigen claims that antibody production may be inhibited by antibiotics as the result of a direct action on the antibody forming tissues.

A third explanation of the relapse phase in the presence of chloramphenicol may be that the type of antibody produced following antibiotic therapy is different in certain respects from normal immune antibody. This could be brought about by some action on the causative organisms with an alteration in the antigenic configuration of the bacterial surface. This explanation however, also appears unlikely since the antibody produced before the antibiotic is given will be normal immune antibody. In addition certain preliminary observations in this laboratory failed to reveal any differences in the type of antibody produced in antibiotic treated animals and control animals injected with typhoid bacilli.

A fourth and more rational hypothesis can be advanced which would seem to explain the above observations more satisfactorily, namely that in some way chloramphenicol increases the number of intracellular organisms. In this way a larger number of organisms would then be available in the reticulo-endothelial tissues to reinvade the blood stream and cause clinical relapse, than in the untreated case. In spite of the fact that intracellular forms of *Salmonella typhi* can remain viable, it is probable that a certain percentage of them are killed off by the cell enzymes. Where treatment with chloramphenicol is continued for 10-14 days the number of intracellular organisms may be decreased sufficiently in this way so that the survivors which may reinvade the blood stream are not present in sufficient numbers to overcome the body's defences and cause relapse in the majority of cases. Where treatment is continued for only three or four days however, there will still be sufficient organisms potentially available to reinvade the blood stream and cause relapse. This hypothesis is difficult of direct proof but does supply an explanation of these high relapse rates in inadequately treated patients in spite of the presence of high levels of demonstrable antibody. Whether chloram-

phenicol does increase phagocytosis in this way is not yet certain but it is well known that other antibiotics such as penicillin and streptomycin may do so under certain circumstances.

The above hypothesis depends for its validity on the fact that phagocytosed forms of *Salmonella typhi* would remain protected from the action of circulating antibiotic. Again however, the available evidence on intracellular protection of organisms from the effect of antibiotics is conflicting. Magoffin and Spink (1951) have shown that intracellular forms of *Brucella* strains are protected from the action of streptomycin. This has also been our experience with streptomycin and *Salmonella typhi* (Watson 1955). Penicillin and terramycin on the other hand appear to be able to penetrate the intact cell more readily. Very little evidence is available concerning the cell penetrating properties of chloramphenicol and this is a problem requiring further investigation.

SUMMARY

In typhoid fever the incidence of relapse may be considerably increased by inadequate chloramphenicol therapy. This does not appear to be dependent on any failure of antibody formation or any alteration in the type of antibody produced. The explanation is put forward that such relapses may be associated with an increase in intracellular parasitism thus creating a greater number of tissue forms able to reinvade the blood stream.

REFERENCES

- ROUS, P. and JONES, F. S. 1916. *J. Exper. Med.* 23, 601.
3. SMADEL, J. E. 1954. *Amer. J. Med.* 17, 246.
6. WATSON, K. C. 1957. *Amer. J. Prof. Med. and Hyg.* (In the Press)
4. STEVENS, K. M. 1951. *J. Immunol.* 71, 119.
5. MAGUFFIN, R. L. and SPINK, W. W. 1951. *J. Lab. Clin. Med.* 37, 924.
6. WATSON, K. C. 1955. *J. Clin. Path.* 8, 55.

JOURNAL VOLUMES

In order to avoid confusion it is felt that it should be explained that Volume 2 of the Journal consisted of three numbers, and that Volume 3 will consist of five.

This situation has arisen because of an effort by the Editorial Committee to terminate the incorrect practice of beginning each number (instead of Volume) at page 1.

It is intended that Volume 4 will consist of four numbers in the normal manner.

PAPER ELECTROPHORESIS OF SERUM PROTEINS IN CLINICAL INVESTIGATION

S. M. JOUBERT

Pathological Laboratory Service, Natal Provincial Administration, Durban.

INTRODUCTION

During the early thirties, Tiselius (1, 2, 3) designed and improved an apparatus to employ the technique of electrophoresis, and applied it to the study of serum proteins. There can be no doubt that this procedure was revolutionary in as much as it became possible to study soluble proteins and characterise them, without apparently modifying their structure. It was a most welcome and desirable advance. The technique employed, however, called for elaborate apparatus and costly and delicate optical accessories and in operation was tedious and time consuming. Of necessity it remained a technique for the fortunate few. The very nature of the procedure, that is, the fractionation of serum proteins in a liquid medium in tubes of capillary dimensions, made further examination of the separated fractions extremely difficult and open to wide experimental error. For general clinical work much greater simplification was necessary.

Tiselius (4) himself sought simplification.

The work of Durrum (5) and Flynn and de Mayo (6) substituted the much simpler and cheaper technique of filter paper as the supporting medium and opened the way for the employment of the technique by almost every clinical laboratory. A formidable literature has grown on the subject and is daily added to.

The purpose of this paper is to relate some observations on the method, techniques and value in clinical application, gained from some 10,000 examinations. The principles underlying the method are refreshingly stressed by Martin and Franglen (7).

APPARATUS

Essential to the procedure is an electrophoretic bath and a D.C. power source supplying a potential gradient across the bath.

1. The electrophoretic bath.

Both the upright or inverted V-type as described by Durrum (5) and Flynn and de Mayo (6) and a flat type of bath have been used with good results. For a 16-hour run on No. 1 Whatman paper, the upright type tends to give more diffused globulin bands, but gives excellent definition of the γ -globulin. On the whole, the flat bath is preferred for haemoglobin and lipoprotein electrophoresis where the narrow bands

are desirable for comparative purposes. The baths must be air-tight, and a buffer capacity of at least 250 ml. per trough is requisite. Platinum electrodes, although more expensive than other non-polarisable electrodes, will save much trouble.

2. The power supply :

The power supply has perhaps been somewhat of an orphan in the published literature, though in practice it makes all the difference between success at reproducibility and indifferent results. In our experience a constant D.C. voltage supply is vital. A set constant D.C. voltage power supply unit as illustrated in figure 1, is preferred to a variable voltage control unit, which tends to vary during the course of a run, except in the rather elaborate and expensive designs.

MATERIALS

1. Paper.

Experience with only Whatman papers has been gained. Nos. 1 and 4 have proved excellent for routine analytical work.

2. Buffer.

The buffer system used and the pH at which the run is achieved, affect the appearance of the final separation, or perhaps more correctly stated, influence the migration rates of the fractions separable on paper. For most clinical applications a barbital buffer of ionic strength 0.06 M and pH 8.6, is suitable. Borate buffers appear to have special applications (Consden and Powell (8)) and the work of Smithies (9) with starch as the supporting medium, amplifies the use of this buffer. Phosphate buffers at pH 8.0 do not give the same resolution as the barbital buffer.

OPERATION

1. Serum.

An unhaemolysed fasting specimen of serum is required and it is good routine practice to run the specimens as fresh as possible, although storage for a week at 4°C. does not appear to alter the fraction if a barbital buffer is used. For routine work prior dialysis against the buffer is unnecessary.

2. Preparation of the paper.

Dry strips 5 cm. wide, labelled with pencil. An application line drawn in pencil is put in position three hours before application is due and allowed to become saturated in position with buffer by the natural capillarity of the paper. In the inverted V-type of bath the paper must under no circumstances be creased over the central rod, as the crease varies the paper matrix and will leave an application mark of unmigrated serum. Under these circumstances saturation equilibrium is established.

The practice of wetting the paper with buffer immediately before application and blotting off excess buffer, introduces an unnecessary variable.

3. Application of the serum.

A considerable number of methods have been described. Some employ direct application from a micro-pipette. This method requires considerable practice if even distribution is to be achieved without scratching the paper. A narrow strip of filter paper soaked in the serum, lightly blotted, and applied to the paper for a few minutes has given good results. Practice in this laboratory is to bevel the one edge of a microscope slide and fashion two slides with the aid of plasticine into a V-shape; the opposing bevelled edges forming a capillary slit. Serum is pipetted into the wide side and evenly layered above the capillary slit by lateral shaking. The capillary edge is then lightly and evenly applied to the paper until the serum has soaked away. The procedure requires a minimum of skill.

In the upright or inverted V-type of bath application is usually done over the suspending rod, which is siliconed to prevent capillary adhesion. Experience gained by this method showed that despite the precaution of siliconing the rod, unmigrated protein at the line of application could not be avoided entirely. Better results were obtained by placing a second siliconed rod adjacent to the suspending rod, applying on this rod and removing it immediately after application.

4. Quantity of serum applied.

If direct application, or application as described, is practised, a rough measure of quantities is necessary. For No. 1 and No. 4 Whatman papers, 0.02 to 0.04 ml. are satisfactory quantities.

5. Time of run.

It is desirable for reproducible results to standardise the time of the run. This naturally depends on the current density and buffer concentration and paper. Using a voltage stabilised at 150 V., 0.06 M. barbital buffer and No. 1 or No. 4 Whatman paper, 16 hours is a satisfactory period. Under these circumstances the current flow per strip is less than 1 milli-amp.

6. Temperature control.

With the small current densities used, the heating effect is minimal. Running the same sample at 4°C. and room temperature with the same equipment, gave no material difference.

METHOD OF EVALUATING THE SEPARATED FRACTIONS

To visualise the end result of an electrophoretic separation of serum proteins, constitutes no problem. After "fixing" the fractions, there are numerous organic dyes which will stain protein and thus render

the separate fractions visible. To have meaning, however, some method of quantitative assessment is vitally necessary. In the classical Tiselius electrophoresis, quantitation of the separated fraction was achieved by the ingenious procedure of refractive index measurements over the length of separation, giving a differential refractive gradient pattern. The area under each curve is proportional to the amount of protein present (assuming protein only to be present). Simple integration gave a quantitative result of tolerable accuracy. Albeit non-specific for proteins, this method put the technique on a firm quantitative basis. As yet there is no such happy ending to paper electrophoresis.

The best approach to the problem is to state an ideal solution.

1. Each fraction must be pure and quantitatively separated after completion of the run.
2. The process of staining must result in each fraction combining with the dye in stoichiometrical proportions and these proportions should not differ in the various fraction, or if they do, these should be capable of accurate determination.
3. Having stained, excess stain should be removable without removing any dye bound to or combined with protein.
4. Methods should be available to assay the amount of dye bound to, or combined with, the protein accurately.

Unfortunately this ideal state cannot be achieved. Work, such as that of Jencks et al. (10), illustrate the thoroughness with which these problems have been examined. In fact, not a single one of the ideal results can be achieved.

Results will vary with the conditions, the dye used and method of assay. It is frankly doubted whether two different laboratories will achieve even reasonable agreement.

What is one to do under these circumstances? Discard the method? The only reasonable approach appears to be to standardise one's own technique thoroughly, establish a range of normality and use the method in a relative or comparative sense.

The method employed in this laboratory is here reproduced.

After completion of the run, the strips are suspended in an oven at 115°C. for exactly 30 min. They are always suspended with the albumin at the lowest end. The strips are then immersed in 1% aqueous acetic acid for 30 mins., dried for 10 mins. at 115°C., and immersed for 20 hours in 0.01% bromo phenol blue solution in 96% alcohol containing 1% acetic acid. After staining for 24 hours, the strips are taken from the staining bath, drained, immersed in a bath of 1% aqueous glacial acetic acid for 20 mins., then rinsed in another bath containing 1% aqueous acetic acid and dried at 115°C. for 10 mins. exactly.

At this stage it is as well to record that initially much more concentrated dyes were used, of the order of 0.1% with various fixatives added. We could not achieve reproducibility with the concentrated dyes. It cannot be over emphasised that these conditions must be adhered to rigourously.

The strips are inspected to ascertain the degree of separation achieved. A good guide is to run a mildly haemolysed adult serum and see whether a haemoglobin band appears between the α_2 - and β -globulin fractions. We consider the clean separation of the α_1 -globulin from the albumin fraction as a good criterion of suitable separation. The line of application should show no unmigrated protein. In many laboratories, the "electrophoretogram" is passed on to the clinician at this stage, probably in the hope, rather than in the belief, that it will mean something. In certain gross disorders it certainly will be of value.

A quantitative estimate of the fractions can be achieved in two ways. Either the specimen is scanned or the fractions are serially or severally eluted and the dye assayed.

In our experience the scanning technique gives reproducible results only if monochromatic light is used. Machines supplying monochromatic light are rather expensive, and an elution technique has advantages if it employs equipment already in use. For this reason the elution technique is recommended.

The bromophenol blue is eluted with 0.1 N Na_2CO_3 for two hours with occasional agitation, after the fractions have been cut out, and the optical densities read at 595 m μ . The individual fractions are expressed as a percentage of total sensitivity or as grams % if a total protein has been done on the specimen.

Excellent reproducibility can be achieved by this method. Running the same specimen from a healthy adult European in conjunction with others for a week, the standard deviations for the fractions were: Albumin, 2.1%; α_1 -globulin, 0.4%; α_2 -globulin, 0.9%; β -globulin, 1.3%; and γ -globulin, 0.8%. If six strips for the same specimen are concurrently run, the standard deviations are somewhat lower. For 50 adult healthy Europeans the average quantities of the various fractions were as follows:

Albumin	53.3%
α_1 -globulin	4.3%
α_2 -globulin	12.4%
β -globulin	13.2%
γ -globulin	16.8%

In this method outlined, it is probable that some albumin is lost due to solubility in the acetic acid. It is assumed that any losses will be constant, within narrow limits, if the standardisation is adhered to strictly.

To speculate on absolute values, however, can serve no purpose, as no standard specimen exists in which the absolute values are known by this technique.

It is practice, however, to check the relative variation of one fraction, viz., γ -globulin. This is done by doing a zinc sulphate turbidity test on all specimens as described by Kunkel (11). Kunkel et al, (12) states that the relationship between turbidity units and absolute value is given by the formula:

$$\text{Gms. gamma-globulin} = (\text{units} \times K_1) + K_2.$$

Based on electrophoretic data (presumable classical Tiselius method) the values of these Constants were found to be $K_1 = 0.053$ and $K_2 = 0.5$.

Using the method described here, the value of K_1 is confirmed as 0.053, but K_2 values are different (unpublished data). For healthy adult Africans $K_2 = 1.0$ and healthy European adults $K_2 = 0.85$. These differences are under investigation; suffice it to state that in routine work the zinc sulphate turbidity converted result is used as a check on γ -globulin determined by electrophoresis. Unless the two results agree closely, it is assumed that there has been a lapse in the standardised technique and the analysis is repeated.

CLINICAL APPLICATION

The method has been outlined, the rigorous standardisation stressed and it has been indicated that the best that can be hoped for is reproducibility in the same laboratory. For the literature-conscious practitioner the wide variations in quoted values must be frightening, if not cause for uncomplementary criticism and perhaps correctly so. However, the method has some definite uses and these are considered to be the following:

A. Uses where diagnostic results can be expected.

1. Nephrotic syndrome.

In this instance a definite pattern is obtained, in our experience, without exception. The albumin is grossly decreased, the α_2 -globulin grossly increased and γ -globulin grossly decreased. All three features must be apparent for diagnosis to be made.

2. In a- or hypogammaglobulinaemia.

In this condition a striking feature is the almost complete absence of gamma globulin. In the four cases which have come to our notice, the albumin fraction was reduced slightly and in one case the α_2 -globulin was raised. As these patients had superimposed infections, it is not clear whether these latter changes are specific.

3. In multiple myelomatosis:

In this condition a striking picture is obtainable if it is not of the so-called normo proteinaemic variety.

Thirty-two patients with this condition have been examined. In three there was no abnormality in the pattern (using the marrow examination to be correct). In two, an abnormal band was present in the β position and in the remainder, the abnormal band was either in the γ or α position. Only the rare macroglobulinaemia can confuse the issue.

B. Non-specific changes associated with disease processes :

In this field quantitative data were anticipated to be most helpful. Unfortunately, many changes are entirely non-specific. This fact has probably limited the value of the procedure more than difficulties in quantitating the result.

One point must be stressed. The method has amply proved the changed albumin-globulin ratio, as elucidated by salting-out procedures, to be almost entirely due to changes in albumin and gamma-globulin concentration. A high total globulin, estimated by salting-out, almost certainly means an increase in gamma-globulin. The α_1 , α_2 and β fractions show little change compared with the gamma-globulin in health and disease.

In brief, one may say that almost any acute or chronic infection in an active stage will decrease the albumin concentration and increase the γ -globulin. Where much tissue breakdown is in evidence, the α_2 -globulin is increased significantly. In parenchymal liver disease the albumin is decreased and gamma-globulin increased, whereas in the established cirrhosis the gamma-globulin is enormously increased. Severe under-nutrition in babies decreases all fractions, although in a large number the α_1 -globulin appears to be increased—whether this is due to the very low albumins encountered, thus giving clearer separation, is not known, but it can frequently be verified.

The most useful application in our experience is in following the course of infective hepatitis. A persistent increase in gamma-globulin is a poor prognostic sign and in many with this trend, frank cirrhosis becomes apparent. In some cases of suspected amoebic abscess of the liver, an increased α_2 -globulin gives the clue. However, it is by no means diagnostic.

The method has been found quite useless in examining peculiarities elucidated by serological tests, odd blood reactions and diseases which appear unusual or unknown or undiagnosable. It is far too crude a method for these purposes.

In malignant disease, changes may be observed, but these are non-specific and appear to affect only the albumin and γ -globulin fractions and the α_2 fraction if there is tissue breakdown or superimposed infection.

Following radio-therapy, changes in these fractions may be observed, but are non-specific.

In comparative studies, whether it be groups or changes in an individual, the method has perhaps its greatest application.

CONCLUSION

The method has been outlined and the difficulties stressed. Clinical application from the point of view of serum proteins is strictly limited.

This, however, does not make the method useless. It has indicated the necessity of re-thinking in many clinical problems. Where do these fractions originate? What controls the quantities present? What is their function? Why is there an apparent inverse relationship between albumin and gamma-globulin?

Moreover, technically there is no bankruptcy as far as improvements are concerned. Ultra-violet assay of the fractions may solve the dye problems. Supporting media more suitable than paper can be found.

There is no reason to believe that the method will not gain in importance as techniques develop.

REFERENCES

1. TISELIUS, A. (1930). *Nova Acta Soc. Sci. upsal.*, Series IV, vol. 7, no. 4.
2. TISELIUS, A. *Trans Faraday Soc.*, 33, 524, 1937.
3. TISELIUS, A. *Kolloidsch.* 85, 129, 1938.
4. CREMER, H. D. and TISELIUS, A. *Biochem. Z.* 320, 273, 1950.
5. DURRUM, E. L. *J. Dm. Chem. Soc.* 72, 2943, 1950.
6. FLYNN, F. V. and DE MAYO, P. *Lancet.* 2, 235, 1951.
7. MARTIN, N. H. and FRANGLEN, G. T. *J. Clin. Path.* 7, 87, (1954).
8. CONSDEN, R. and POWELL, N. M. *J. Clin. Path.* 8, 150 (1955).
9. SMITHERS, O. *Biochem. J.* 61, 2, 629, 1955.
10. JEUCHS, W. P., JETTON, M. R. and DURRUØ, E. L. *Biochem. J.* 10, 205, 1955.
11. KUSCHEL, H. G. *Proc. Soc. Exp. Biol. and Med.* 66, 217, 1947.
11. KUNKEL, H. G. *Proc. Soc. Exp. Biol. and Med.* 66, 217, 1947.
12. KUNKEL, H. G. AHRENS, E. H. *Eismonger, W. J., Gastroenterology.* 11, 449, 1948.

ELDON CARDS

R. L. PINDER

Durban Blood Transfusion Service

(Abstracted from *British Medical Journal*, Volume 2, No. 5003,
24th November, 1956)

Dr. Kund Eldon of the Nordsik Insulinlaboratorium, Gentofte, has published an article on the "cards for simultaneous ABO and rhesus determinations", which are known as "Eldon cards".

These cards have been used extensively in Denmark since February 1955 and it is estimated that some 60,000 have been used to date.

The card has four panels covered with regenerated cellulose. On each panel is dried 0.06 ml. of reagent (100 ml. reagent is composed of

10 ml. serum, 90 ml. 6% dextran, and 2 drops of 5% heparin solution). The first panel has on it Anti-A, the second panel has on it Anti-B; these are for estimating the ABO group of the patient. The third panel has on it Anti-D for estimating the Rhesus type of the patient. The fourth panel has "neutral" serum, i.e. serum from a group AB person, which contains no antibodies. This is used as a negative control.

Moulded plastic sticks and a controlled dropper pipette are supplied with each set of cards.

These cards are simple to use and effective *provided the instructions are followed implicitly*. They are used at room temperature but must be stored at less than 30°C.

Two techniques may be employed:—

- (i) for capillary blood;
- (ii) for blood collected into an anticoagulant.

N.B. Do not use acid-citrate-dextrose as the anticoagulant for this inhibits the agglutinations.

TECHNIQUE I

Holding the pipette provided perpendicularly, add one drop of tap water to each panel. Dissolve reagents by stirring with the flat end of the plastic sticks provided. It is advisable to use one stick per panel to avoid contamination. After use, wash and dry the plastic sticks with care to remove all the reagent.

A "hemisphere" of blood is removed from an earprick by touching the freshly expressed capillary blood with the flat end of the stick. Mix the blood with the dissolved reagent on panel 1, and spread the mixture over the whole panel. Repeat the procedure with the other three panels, using separate plastic sticks for each one to avoid contamination.

Leave the card for one minute, fill in the patient's name, age, etc., on the card. Then tilt the card slowly—about four times in three minutes—allowing the mixture to slide from border to border in the panel.

Read result. Allow card to dry. When thoroughly dry, the cards can be preserved with a coating of clear varnish and will then become a permanent record of the actual test.

TECHNIQUE II

For blood collected into an anticoagulant (other than acid-citrate-dextrose). To twelve drops of saline add two drops of packed cells. Holding pipette at an angle of 45°, place one drop of the mixture onto

each panel. Using the plastic sticks, stir to dissolve reagent and mix thoroughly with the cell suspension. Leave the card on the bench for one minute, during which time the patient's name, age, etc., should be noted on the card. Tilt the card back and forth for one minute. Read results and then allow the card to dry.

READING				RESULTS
Anti A	Anti B	Anti D	Control	Group
Agglutination	No agglutination	No agglutination	No agglutination	A Negative
Agglutination	No agglutination	Agglutination	No agglutination	A Positive
No agglutination	Agglutination	No agglutination	No agglutination	B Negative
No agglutination	Agglutination	Agglutination	No agglutination	B Positive
No agglutination	No agglutination	No agglutination	No agglutination	O Negative
No agglutination	No agglutination	Agglutination	No agglutination	O Positive
Agglutination	Agglutination	No agglutination	No agglutination	AB Negative
Agglutination	Agglutination	Agglutination	No agglutination	AB Positive

If agglutination is observed in the control panel, then the results are meaningless; this can occur for example in the presence of autoagglutinins, bacterial action on the cells and in some protein disorders.

A negative result in the Anti-D panel means that the patient must receive rhesus negative (cde cde) blood. These cards are inadequate for testing blood meant for transfusion.

Of the 60,000 cards used in Denmark there were two errors in the ABO groups; this was found to be due to bad technique. The patients were of the group A₃ and the weak agglutination in the first panel was masked by the use of too thick a cell suspension. There were four rhesus errors, again due to bad technique. There were twenty cases where results were doubtful, thirteen of which were due to misapplied technique and the remaining seven were due to the cells being abnormally agglutinable, giving a total error of 0.04%.

Dr. Eldon concludes by saying "the results give the impression that to be of use, the method must be closely adhered to and the directions followed carefully."

These cards are being distributed by the Natal Provincial Pathologist to the Provincial hospitals. It is hoped that their use will enable the patient to be given the correct group of blood, which will hasten his recovery, and also stop the continuous drain on group O blood, the supply of which is limited, and which must not be regarded as "universal donor" blood.

THE SUBTERRANEAN TERMITE

FRANK E. CHEEK

The statements here recorded are the results of practical experience only, but may nevertheless be found to be of real value, particularly in connection with any proposed building construction.

The common name for this termite is the White Ant. From September to March is the swarming season, and in this period the flying ants (potential Kings and Queens) emerge from the earth, pair off, and, if they escape their numerous enemies, re-burrow for about two inches into the ground. The process takes place after rain, when the ground is soft. Once they are back in the soil, they cover themselves with a crust of soil and copulation takes place. After six weeks the colony consists of about 200 young ants, and since these are nearly white it is thought to be from them that the term "White Ants" is obtained. The young are divided into "workers" and "soldiers". The former are a deep cream colour but have a darker head; whereas the latter, although having a similarly coloured body, have a much larger dark head with pronounced mandibles for use in defence of the colony. At this stage, the Queen is still a flying ant, but by the time the workers have matured, burrowed deeper into the earth, made a cell for the Queen and surrounded it by a fungus "garden", the Queen starts to develop the enlarged abdomen which eventually reaches the size of an adult's index finger. During this process, other workers enlarge the inside and outside of the Queen's cell. It has been claimed that new chambers are built, of increasing size, to accomodate the growing Queen, but this is unlikely since Queen cells of varying sizes in the one colony are not found. A cell frequently contains more than one Queen but never more than one King. If a King and Queen are separated, the Queen will remain stationery until the King returns.

In crevices near the fungus "garden", the eggs of the ants are placed to permit facility of access by newly hatched ants to the fungus. The probable number of eggs deposited daily is about 30,000 although figures as high as 80,000 have been quoted by some authorities. The eggs are deposited in a liquid which rapidly crystallizes.

The life of a Queen is about seven years, at the end of which time, the ant shrinks and becomes very dark brown. An outstanding authority has stated that the workers then consume the Queen. A potential Queen is then selected from among the "workers", but it is doubtful whether a potential Queen is without sex and sight as are normal "workers" and "soldiers", although it has been claimed that these attributes are acquired by the potential Queen after selection. Although Queens lose the power of flight when they are developed, Kings do not do so; and, being able to move in and out of the Queen cell, are frequently found to be absent

when nests are opened. If the Queen cell is found to be empty, it is because it has been opened after the consumption of one Queen and before the instalment of another. On opening a nest, the colony may be found to be extinct ; this is often due to disease.

The mushroom-like fungi appear as objects about the size of a pinhead. They die within 24 hours if exposed to moving air.

Three different species of White Ants cause damage to property in Natal.

The depth of the nest from the surface depends largely on the hardness of the soil and in the Durban area it varies from five to nine feet. One, 28 feet down, was found in this area. If, however, the nest is thought to be deep under a house, gassing is employed. Despite the high moisture content of ants, they can survive with very little water. The termites have been known to operate up to 75 yards from their nest, but the distance is usually less than this, despite claims of half a mile by some authorities.

There are several methods of extermination, but manual removal is considered to be the best. Other methods are gassing, the application of an insecticidal powder, and the use of poisoned bait.

It is believed that although no preventative measures will prevent entry to property by White Ants, the use of a suspended floor and anti-guard will enable the termites to be detected and exterminated with maximum ease. Other methods of prevention are pressure-treated timber, impregnation of wood or soil with pentachlorophenol, the sprinkling of surface with salt, the use of an ash and sand mixture (in which "runways" can not be built), and the sprinkling of a surface with lime. None of these methods is reliable, however.

Synopsis of a lecture delivered to the Natal Branch of the Society.

ABSTRACTS

The preservation of bacteria. Greaves, R. I. N. (1956). *Canad. J. Microbiol.*, 2, 365.

Details of an apparatus for drying bacterial suspensions, with details of temperature and the substrate giving maximum survival.

Unusual bacteriological and serological results in patients suffering from typhoid or paratyphoid fever. Simon, I. (1956). *Arztl. Wschr.*, 11, 283.

Stated that unusual results in the above conditions are obtained as a result of antibiotic therapy. Causative organisms may sometimes not be isolated, or only atypical colonies. Agglutinins may be demonstrable in the absence of detectable *Salmonellae*, or *Salmonellae* may be demonstrated in the absence of agglutinins.

On the use of tetrazolium blue antigen and centrifugation in the Brucella abortus ring test. Genest, P.; Lahaye, L. and Filion, R. (1956). *Canad J. Comp. Med.*, **20**, 86.

Comparisons of methods for the detection of brucellosis using 897 herds for one comparison, and 1,170 herds for another.

Further experiments with combinations of pancreatin and Q.A.C. for cultivation of M. tuberculosis. Saxholm, R. (1955). *Amer. Rev. Tuberc.*, **72**, 98.

Using 3,818 sputum specimens, favourable comparison was found between sodium hydroxide and a mixture of pancreatin and the quaternary ammonium compound "desogen".

Blood media for cultivation of Mycobact. tuberculosis. Tarshis M. S.; Parker, M. V. and Dunham, W. B. (1955). *Acta. Tuberc. Scand.* **31**, 92.

A comparison of media for the cultivation of tubercle bacilli incorporating penicillin, malachite green or sodium tellurite.

Egg-free and egg-containing solid media for the cultivation of tubercle bacilli. Kukherm, B. and Danecke, K. (1955). *Zbl. Bakt., I. Abt. Orig.* **163**, 541.

A description of a modification of Hohn's medium, in addition to a description of an egg-free medium containing, among other things, brain extract and caseine peptone.

Comparison between the media of Sula and Löwenstein-Jensen. Janowiec, M. and Tuszyńska, B. (1955). *Gruzlica*, **23**, 771.

The value of the solid medium of Löwenstein-Jensen, compared with the liquid one of Sula, appears to depend on the use to which it is put.

The continuous culture of bacteria. Herbert, D.; Elsworth, R. and Telling, R. C. (1956). *J. gen. Microbiol.* **14**, 601.

A thorough mathematical treatment of the considerations involved in the continuous culture of bacteria, and details of a series of experiments to assess correlation of practical and theoretical results.

A new aerobic and anaerobic control medium for sterility tests. Glausen, O. G. (1956). *Acta. Path. Microbiol. Scand.*, **38**, 107.

Comparison of a modification of Bonnel's hydrosulphite medium with the standard thioglycollate medium of the United States Pharmacopoeia.

The survival of micro-organisms in culture media. Engley, F. B., Jr. (1956). *Texas Reps. Biol. Med.*, **14**, 114.

A short discussion on the factors influencing survival of micro-organisms *in vitro*, and 26 tables summarizing the previously published results of many workers.

PATHOLOGIST'S LETTER

Electrophoretic studies of haemoglobin have led to further understanding of haemolytic anaemias of the inherited types. Other methods of study are resistance to alkali, solubility in the reduced state and chromatography.

Three forms of haemoglobin, Adult—A, Foetal—F and Sickie—S have been studied for some time. Persistence of varying amounts of foetal haemoglobin in post-partum life is the determining factor in Thalassaemia⁽¹⁾, whether it be the trait or the fully developed Cooley's anaemia. The Sickie cell trait is easily recognised in wet preparations of blood when the haemoglobin is reduced. Here again the homozygous or heterozygous forms determine the severity of the condition. Six further types of haemoglobin are now defined, i.e. G, D, E, C, H, and I⁽²⁾. The difference is in the globin part of the molecule.

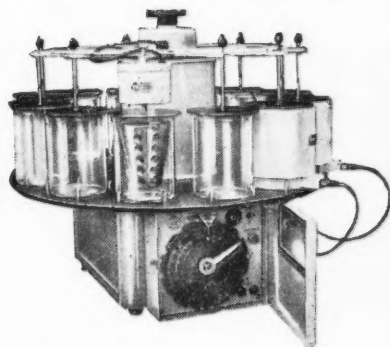
The methods of study have been well described by White, J. C. et al⁽³⁾, and Huisman T. H. J. et al⁽⁴⁾. It is well-known that persons possessing the trait for these forms of haemoglobin do not necessarily suffer from anaemia. However, in several instances, the occurrence of mixed types of haemoglobin have brought patients to the haematologist. The following mixed types are described: Thalassaemia—Haemoglobin—D—Disease⁽⁵⁾, Sickie Cell—Thalassaemia Disease or Micro-drepanocyte disease,^(6 7 8) Sickie Cell—Haemoglobin—C—Disease⁽⁹⁾, Thalassaemia—Haemoglobin—E—Disease⁽¹⁰⁾, Sickie Cell—Haemoglobin—D—Disease⁽¹¹⁾, Thalassaemia—Haemoglobin—C—Disease⁽¹²⁾.

These studies are of great interest to ethnologists and in this regard the surveys of Raper, A. B.⁽¹³⁾ and Lehman, H. and Raper, A. B.⁽¹⁴⁾ have shown that persons with the sickle cell trait are more resistant to malarial parasites. These abnormal haemoglobins are not unknown in South Africa, and it is as well that technologists should be aware of the progress made in our understanding of these haematological conditions.

REFERENCES

1. SINGER, K. ET AL. 1951. *Blood*. 6. 413.
2. RUCKNAGEL, D. L. ET AL. 1955. *Blood*. 10. 999.
3. WHITE, J. C. ET AL. 1956. *Paper Electrophoresis. A Ciba Foundation Symposium J. A. Churchill, Ltd., London.* 43.
4. Huisman, T. H. J. ET AL. 1955. *L. Lab. C Clin. Med.* 46, 2, 255.
5. HYNES, M. AND LEHMAN, H. 1956. *B.M.J.* 2. 923.
6. SINGER, K. ET AL. 1955. *Blood* 10. 5. 405.
7. STURGEON, P. ET AL. 1952. *Blood* 7. 350.
8. SINGER, K. AND CHERNOFF, A. I. 1952. *Blood* 7. 47.
9. HAYS, E. F. AND RALPH, L. E. 1955. *Annals Int. Med.* 43, 2. 412.
10. CHERNOFF ET AL. 1954. *Science* 120 605.
11. ITANO, H. A. 1951. *Proc. nat. Acad. Sci. Wash.* 37. 775.
12. SINGER, K. ET AL. 1954. *Blood* 9. 1032.
13. RAPER, A. B. 1956. *B.M.J.* 1. 956.
14. LEHMAN, H. AND RAPER, A. B. *B.M.J.* 2. 333.

M.S.E.
HISTOKINETTE



S.A. Agents and Stockists:

MACDONALD ADAMS & Co.

JOHANNESBURG
P.O. Box 68 - Phone 238161

D U R B A N
P.O. Box 1807 - Phone 60888

READERS' FORUM

The Editor,
Sir,

It is felt that some comment might reasonably be made on the report on the committee meeting of the Pathologists' Group of the Medical Association to consider the registration and training of medical technologists. The report was circulated to those present at the Annual General Meeting for 1956 of the Natal Branch of the Society. Since the report was made by the Secretary of the Society, it has, by now, presumably been circulated to the other Branches and been placed in a position for discussion.

On the subject of registration, it is, perhaps, well to point out that Dr. Louw, who is reported to have stated that the public do not employ medical technologists, is apparently taking a very restricted view of the employment situation. Medical technologists are employed privately by certain pathologists; and commercial firms employ technologists for the control and testing of their medical products. Surely both these groups of medical technologists can be said to be employed by the public.

The belief, reported as having been voiced by Dr. Thomas, that technologists in diagnostic medical laboratories should be registered, while registration should be withheld from other technologists, would seem to be making an unnecessarily fine distinction. It would seem distinctly anomalous if a technologist engaged in reporting the isolation of a *Salmonella*-like organism should be eligible for registration, while another in a laboratory situated in the next room who is naming that organism should be ineligible because his work is not diagnostic. If a distinction should have to be drawn in the future between technologists liable for registration and those not liable, it is hoped that the distinction will not be that of diagnosis. It might well be mentioned that if a *Salmonella* had already been isolated from the patient who produced the one cited in the above example, the technologists performing the second isolation would not be engaged in a diagnostic procedure. Much of the work performed in a pathology laboratory is not for the purpose of diagnosis, and this strengthens the argument that diagnosis is not adequate as a criterion for the assessment of the work of a laboratory with a view to its recognition or failure to be recognised as a place in which the technologists may be registered.

It is reported that it was felt that there is a definite need for workers to do the purely mechanical routine of the laboratory. The naming of such workers appeared to rest on whether they would aid or assist technologists: a distinction best left to classical scholars; since it is very doubtful whether the average technologist would be interested in the difference! It might be interesting to know what the committee considered was the difference between an aide and an assistant. The point could surely have been left until a much later date. In any case,

technical assistants to technologists already exist in many laboratories, possibly as "aides" or something else in some. However, the principle of such appointments is reported as being their use for conducting "limited laboratory procedures". No definition of this term was apparently attempted and it could be used at will by indiscriminate persons to secure the running of a service at lowered costs (presuming, of course, that they are paid less than technologists). In any case, the reported opinion that such persons would enable a student technologist to acquire a better training, is open to grave doubts. It is felt most unlikely that such would be the case, if anything, rather the reverse. The general effect on technologists would be likely to be a reduction in their efficiency, a reduction in the fluency of their technique and a false sense of superiority.

The suggestion that the whole of medical bacteriology, mycology, parasitology as well as all branches of veterinary microbiology in addition to the subject of food microbiology should be squeezed into one subject: microbiology, to be of an equivalent standing to histopathological technique, is to lose all sense of proportion. The field of bacteriology alone is wider than that of histopathological technique. This suggestion must obviously be reconsidered.

The standard of instruction and examination (taking that at present in use by the Natal Branch as a comparison) will have to be very considerably raised before the possession of one of the proposed certificates will, if equation with the degree of B.Sc. is contemplated, evoke anything other than subdued mirth in academic circles. It is generally accepted that the diploma of the South African Pharmacy Board is more nearly equivalent to a bachelor's degree than anything at present issued by the Society, and anyone who has experienced any part of the training of a pharmacist will agree that the Society and its instruction and examinations have a great deal of room for improvement.

It is, perhaps, unfortunate that the presentation of the report left so much to be desired. It would be interesting to learn the name of the publication, to paragraph 10 on page four of which Dr. Murray is cryptically reported to have drawn the meeting's attention. Although no mention is made of it before or afterwards, our attention is suddenly arrested by the statement that "the method of training should be a compromise of B and C"!

The report ends with the comments of the Secretary of the Society. The fact that there is not even a pretence at unbiased presentation of arguments for and against the proposals of the reported meeting, is to be deplored. It cannot be considered correct for the Secretary of a national society to voice personal opinions when acting as a spokesman for that society. The opinions were, it is felt, sound, but that does not detract from the very dubious ethics of their presentation.

It is agreed that it would be better if veterinary, medical and food technologists could belong to one necessarily more powerful society. The fact that the Society as it is at present constituted would lose its

identity is necessary, incidental and of no consequence. Its death-agonies would be the birth-pains of a bigger and better organisation. However, it is difficult to see how societies formed by the other groups which it is hoped to include with medical technologists, would be in any way unpleasantly competitive. The fields are all well defined although possessing similar pasturage. In any case, what do *they* think about it?

Yours faithfully,

AN INTERESTED TECHNOLOGIST.

The Editor,

Dear Sir,

In the September issue of your journal, page 17, a letter appeared by Mr. G. W. Wikeley under the section of the "Readers' Forum".

I feel it necessary to help Mr. Wikeley in regard to certain facts pertaining to the training of Medical Technologists in South Africa. For him to say that "in Natal where the original training course is the oldest recognised course in the Union", is absolutely incorrect.

Forty years ago in 1917 the South African Institute for Medical Research selected the first four matriculated young men for training in Medical Laboratory Technique (now Medical Technology). These four trainees were selected from more than a hundred applicants to be "apprenticed" for three years during which time they were given a systematic course of lectures in the relevant subjects as specified to-day and in addition it was necessary for us to attend evening classes in Physics and Chemistry at the Old "School of Mines" (now Witwatersrand University). Lectures in Physiology and Anatomy were given by Dr. Heberden and the standard was approximately the same as that of today.

It may be of interest to our Natal friends to know that Mr. F. H. Joseph, well known to those who have been trained in Natal, was our first lecturer. He was succeeded by Dr. Harvey-Pirie who in 1918 became our first Routine Superintendent.

Though there was at that time no supervision of the Syllabus and registration of Technologists by the Medical Council, nevertheless examinations were written and results kept for Institute records. It was, therefore, an organised and recognised course and incidentally the only one of its kind in the Union. The "apprenticeship" or training scheme was discontinued in 1930 and only revived just before the last war.

As one of the first four individuals to have been trained in Medical Technology in South Africa, I feel proud to be able to record these few historical facts in the hope that they might prove of interest to readers of your Journal.

Yours faithfully,

P. Roux.

The Editor,
Sir,

It occurred to me that some of your readers might, through the pages of this Journal, be able to solve two problems which I have encountered.

The first is a practical technique for the manipulation of glass wool. Some specimens of glass wool are of coarse texture and these are particularly brittle and difficult, as well as dangerous, to handle without (or even with) rubber gloves. Such a wool is that of the firm of E. Merck of Darmstadt. The problem is an old one, but re-presented itself to the writer whilst attempting to produce a plug of glass wool about two inches in length in a piece of glass tubing of internal diameter of approximately one quarter of an inch, for use as a filter to prevent the contamination by air of cultures grown by the cellophane-sac technique.

The second conundrum is simply this : why do the rubber liners in the screw-caps of glass bottles, e.g. "Universal" containers, become distended into the bottle and frequently rupture or even completely lose an approximately circular central portion, when the bottles in question contain ether?

Yours faithfully,

PETER N. BUCK.

THE CENTRAL AFRICAN ASSOCIATION OF MEDICAL LABORATORY TECHNOLOGISTS

(Salisbury and District Branch)

PROGRAMME FOR THE FIRST 6 MONTHS OF 1957

- January 30th* : "Research into Soil Erosion." by Mr. D. C. Jackson.
February 27th : "The value of Counterbalanced Appliances in Orthopaedic Surgery." by Mr. E. J. Nangle. This talk will be accompanied by a film.
March 27th : "Clinical Interpretation of Pathological Reports." by Dr. G. M. Woodward.
April 24th : "The History and Administration of the N.I.D. Hospital." by Mr. S. Kennedy. This lecture will be held at the N.I.D. Hospital.
May 29th : Quiz.
June 26th : "Radiotherapy in Non-malignant Conditions." by Dr. A. Grieg.

J. P. COATES PALGRAVE,
Hon. Secretary.

NOTICE TO CONTRIBUTORS

All contributions are to be addressed to:—The Editor, The South African Journal of Medical Laboratory Technology, Room 213, Dept. of Pathology, Medical School, Umbilo Road, Durban.

Contributions may be written in English or Afrikaans, and should preferably be typed in double-spacing on foolscap sheets on one side of the paper only.

Figures should be drawn in Indian ink, and all figures and tables should be labelled as such (e.g. Figure 1, Table 1, etc.).

Authors should make adequate references to previous works on their subjects. These should be set out as follows:—Author's surname and initials of Christian names; the year of publication (in parentheses); the name of the journal, which should be abbreviated according to the World List of Scientific Periodicals (see below); the volume number (underlined); and the first page reference.

Example:—Moron, I. B. (1960). J. unsuccess. Med., 20, 99. References to books should give the author's name and initials, the year of publication, title of book, name of publisher, and town in which published.

References should be arranged in alphabetical order of the authors' surnames. If more than one work by the same author is listed, these should appear in chronological order.

Technologists are reminded that regulations demand that all original articles of a technical or scientific nature must be approved by the heads of their departments before being submitted for publication.

Title abbreviations according to World List of Scientific periodicals

All nouns commence with capital letters, and adjectives small letters. Articles, conjunctions and prepositions are omitted.

Examples:—

<i>J. Amer. med. Ass.</i>	<i>S. Afr. J. clin. Sci.</i>
<i>Lancet</i>	<i>Stain Tech.</i>
<i>Amer. J. clin. Path.</i>	<i>J. Bact.</i>

REPRINTS AND PHOTOGRAPHS

If requested before publication, 24 reprints of original articles will be supplied free to contributors. As a temporary measure, contributors are asked to defray the costs of publishing diagrams and photographs accompanying articles.

KENNISGEWING AAN INSENDERS

Alle bydrae moet as gevolg geadresseer word: Die Editor, Die Suid Afrikaanse Joernal van Mediese Technologie, Kamer 213, Dept. van Patologie, Mediese Skool, Umbiloweg, Durban, Natal.

Bydrae mag in Engels of Afrikaans geskryf word en moet verkieslik getik wees dubbel spasiering op folio-papier en net op een kant van die vel.

Figure moet in Indiese ink geteken word en alle figure en tabelle moet geteikener word as sulks (b.v. Figuur 1, Tabel 1, ens.).

Auteurs moet voldoende referensies gee tot vorige werke oor hulle onderwerpe. Die moet as volg uiteengesit word:—Auteur se familie-naam en voorletters; die jaar van uitgawe (in hakies); die naam van die Joernaal, wat moet verkort volgens die Wêreld Lys van Wetenskaplike Tydskrifte (sien hieronder) die volume nommer (onderstreep); en die eerste pagina referensie.

Voorbeeld:—Moron, I. B. (1960). J. unsuccess. Med., 20, 99. Referensies tot boeke moet die auteur se naam en voorletters meld, die jaar van uitgawe, titel van boek, naam van uitgewer, en stad waar dit gepubliseer is.

Referensies moet in alfabetiese orde, volgens auteurs se familienaam gerangskik word. Indien meer dan een werk deur dieselfde outeur gemeld word, moet dit in tydsorde voorkom.

Tegnoloë word daaraan herinner dat regulasies vereis dat alle oorspronklike artikels van tegniese of wetenskaplike aard moet die goedkeuring dra van hulle departementale hoofde voor dit ingestuur word vir publikasie.

Titel verkortings volgens Wêreld Lys van Wetenskaplike Tydskrifte

Alle selfstandige naamwoorde moet begin met hoofletters en byvoeglike naamwoorde met klein letters. Artikels, verbindings, en voorsetsels word uitgelaat.

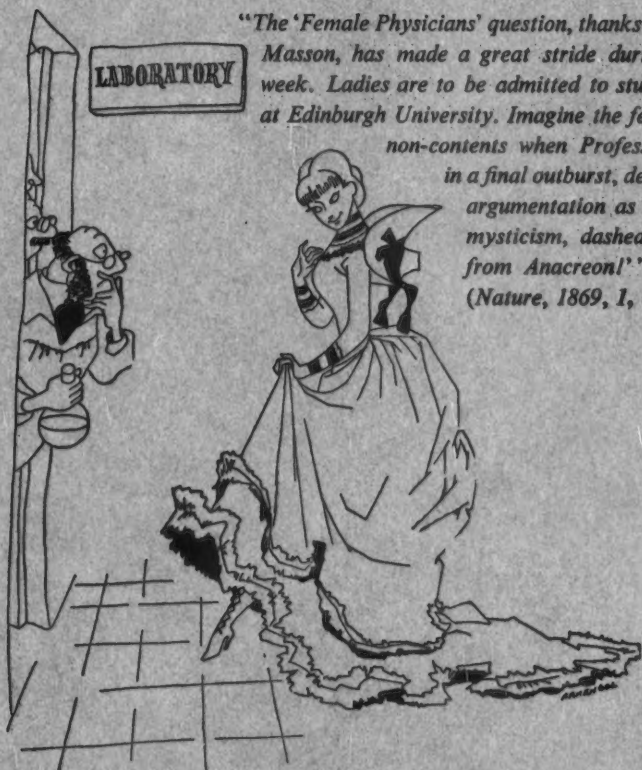
Voorbeelde:—

<i>J. Amer. med. Ass.</i>	<i>S. Afr. J. clin. Sci.</i>
<i>Lancet</i>	<i>Stain Tech.</i>
<i>Amer. J. clin. Path.</i>	<i>J. Bact.</i>

HERDRUKKE EN FOTOGRAWE

Indien aanvraag ingedien word voor publiser, sal 24 herdrukke van oorspronklike artikels vry aan beydraers verskaf word. As in tydelike maatregel word bydraers gevra om die koste van publiser van fotos en tekeninge wat saam met artikels gaan self te betaal.

Ladies are to be admitted



"The 'Female Physicians' question, thanks to Professor Masson, has made a great stride during the past week. Ladies are to be admitted to study Medicine at Edinburgh University. Imagine the feelings of the non-contents when Professor Masson, in a final outburst, described their argumentation as 'rampageous mysticism, dashed with drivel from Anacreon!'"
(Nature, 1869, I, i, 25)

In 1869 scientific clinical methods were nearly as unpopular as women doctors. Today men and women work together in clinical, bacteriological and pathological laboratories all over the world; and in more than seventy countries regard the products of the B.D.H. Laboratory Chemicals Group with equal favour.

B.D.H. LABORATORY CHEMICALS

THE BRITISH DRUG HOUSES LTD. B.D.H. LABORATORY CHEMICALS GROUP POOLE ENGLAND

Agents in South Africa: MACDONALD ADAMS & CO., JOHANNESBURG and DURBAN

SAP/LC/13

When you want to control OEDEMA
think first of . . .



Diamox*

Acetazolamide Lederle

A nonmercurial oral diuretic. Acts by inhibiting the enzyme carbonic anhydrase. Produces prompt, ample diuresis lasting from six to twelve hours. Morning dosage allows an uninterrupted night's sleep. Well-suited to long-term use. Nontoxic.

The most widely prescribed drug of its kind!

Indicated in cardiac oedema, epilepsy, acute glaucoma, pre-menstrual tension, oedema associated with toxæmia of pregnancy and oedema caused by certain types of electrolytic imbalance. Offered in scored tablets of 250 mg. for oral use.

PACKED IN BOTTLES OF 25 AND 100

* Regd. Trade Mark

LEDERLE LABORATORIES DIVISION

AMERICAN CYANAMID COMPANY

PEARL RIVER NEW YORK



Sole South African Distributors:

ALEX LIPWORTH LIMITED

JOHANNESBURG

CAPE TOWN

DURBAN

